IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Attorney Docket No. 071007/0137

In re application of:
BHATTACHARJEE *et al.*

Group Art Unit: 1641

Serial No.: 08/886,044

Examiner: S. Devi

Filing Date: June 30, 1997

For: *VACCINE AGAINST GRAM-NEGATIVE BACTERIAL INFECTIONS*

DECLARATION UNDER 37 CFR §1.132

Assistant Commissioner of Patents
Washington, D.C.

Sir:

I, Dr. S.E. Greisman, hereby declare and state as follows:

1. I have been involved in research on endotoxin for over 30 years and have published extensively in highly respected journals such as *The Journal of Experimental Medicine* and *The Journal of Clinical Investigation*. I have also received the highest award given to investigators involved in endotoxin research by the International Endotoxin Society, i.e. Honorary Lifetime Membership. Most recently, I published an extensive, critical review of the literature in the *Journal of Endotoxin Research* that addressed the question of whether antibodies to the inner core region of Gram-negative bacterial endotoxin may be useful for the treatment of sepsis. A copy of this article, "Evidence Against the Hypothesis that Antibodies to the Inner Core of Lipopolysaccharides in Antisera Raised by Immunization with Enterobacterial Deep-rough Mutants Confer Broad-Spectrum Protection During Gram-Negative Bacterial Sepsis," is appended.

2. It is abundantly clear that the literature in the field of endotoxin research pertaining to the efficacy of immunotherapy for treatment of endotoxemia or of Gram-negative bacterial sepsis is quite contradictory. Despite the publications by Ziegler, Braude and colleagues that antisera to the inner core region of *Escherichia coli* O111 (Rc mutant J5, hereinafter "the J5 mutant") successfully treated both animals and patients with sepsis, subsequent investigators have been unable to reproduce the positive experience with J5 antisera or with antisera to other inner core epitopes of LPS (e.g. R595 epitopes). The concept that antisera to Rc (J5) and Re (R595) rough mutants do not provide significant broad-spectrum protection during Gram-negative bacterial sepsis is supported by the failure of 5 clinical trials to demonstrate protection by antisera or immune globulin fractions. All of these trials were reported after Ziegler's clinical study. In the first negative trial (performed by Braude and Ziegler's group) pre- and post-immune J5 antisera were given prophylactically to patients with neutropenia; there was no difference in rates of Gram-negative bacteremia, febrile episodes or mortality (McCutchan JA, Wolf JI, Ziegler EJ, Braude AI. *Schweiz Med Wochenschr* 1983;113 [Suppl 14]:40-45). In the second negative clinical study, a gamma globulin fraction prepared from donors with elevated antibody titers to J5 LPS proved no more effective than fractions with lower titers in preventing Gram-negative bacterial infections when given immediately before induction of aplasia in patients with leukemia (Lecomte F, et al. *Presse Med* 1989;18:1419). In a third trial, an IgG fraction prepared from the sera of volunteers immunized with the *E. coli* J5 mutant bacterial vaccine provided no more protection against mortality when given to patients in septic shock than did the IgG fraction prepared from standard plasma pools. Furthermore the J5 immunoglobulin did not reduce the number of

systemic complications of shock and did not delay the occurrence of death from systemic shock (Calandra et al, *J Infect Dis* 1988;158:312). In a fourth trial, infusions of human immunoglobulin preparations selected for their high content of IgG to R595 LPS afforded no greater protection against subsequent Gram-negative bacterial infections or their systemic complications in patients at high risk after major surgical procedures than did comparable immunoglobulin preparations containing on average 7-fold lower amounts of anti-R595 IgG (The Intravenous Immunoglobulin Collaborative Study Group. *N Engl J Med* 1992;327:234). In a fifth trial, 73 children with severe infectious purpura, the majority secondary to *N. meningitidis*, received J5 immune or pre-immune plasma. The anti-J5 plasma did not affect the clinical course, the rate of decrease of TNFα or IL-6 or mortality (J5 Study Group, *J Infect Dis* 1992;165:695).

Similarly, it has been difficult for many investigators to demonstrate the protective capacity of J5 antisera in the laboratory. For example, after many attempts I have been unable to demonstrate the beneficial effect of J5 antisera even when administered prophylactically in my own laboratory against either endotoxemia or Gram-negative bacterial sepsis (Greisman SE and Johnston CA. Failure of antisera to J5 and R595 rough mutants to reduce endotoxemic lethality. *J Infect Dis* 1988;157:54; Greisman SE, DuBuy JB, Woodward CL. Experimental gram-negative bacterial sepsis: Reevaluation of the ability of rough mutant antisera to protect mice. *Proc Soc Exper Biol Med* 1978;158:482). Further, as I document in my recent 1997 review, the demonstration by others that the administration of J5 LPS or whole boiled J5 bacterial vaccine can protect animals under specific experimental conditions has not suggested any approach that would work for human subjects under clinical conditions nor the mechanism

of such protection. For example, it is not feasible to administer an endotoxin-containing vaccine intravenously or to give immunizations frequently over several months for the prophylaxis of sepsis. Thus it is not obvious from the current state of the art that a J5 vaccine can be made for effective prevention or treatment of Gram-negative bacterial sepsis nor the mechanism by which such a vaccine might be protective. While it is my personal belief, based on my own research and my review of the literature, that an anti-endotoxin vaccine for effective broad spectrum therapy of sepsis is unlikely to be developed, as a scientist I am willing to accept data from properly controlled experiments that demonstrate the efficacy of such a vaccine provided that the mechanism of such efficacy is shown to be related to the immune response to the LPS portion of the vaccine.

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I hereby declare that all the statements made herein of my known knowledge are true and that all statements made on information and belief are believed to be true; and further, that these statements are made with the knowledge that willful false statements are so made punishable by fine or imprisonment, or both, under Section 101 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

March 16, 2000
Date

Sheldon E. Greisman, M.D.
S.E. Greisman